ENZYMATIC CONVERSION OF WASTE OIL TO BIODIESEL IN A SOLVENT-FREE SYSTEM

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Introduction

Production of biodiesel from vegetable oil and animal fats has drawn more and more attention because of the increasing awareness of environmental pollution and short supply of fossil fuels. Presently, the industrial-scale production of biodiesel is performed chemically, using alkali as catalyst. However, there are several problems with this process, such as excessive methanol requirement, high energy demanding, difficulties in glycerol recovery, disposal of fatty acid alkaline salts (soaps) creating other environmental concerns. Thus enzymatic alcoholysis of triacylglycerols (TAG) seems to be a promising alternative because of its mild reaction conditions, easy recovery of glycerol and being free of chemical wastes.

Several studies on lipase-catalyzed alcoholysis of vegetable oils and animal fats with primary and secondary alcohols in a solvent or a solvent-free system have been reported.[1-3] Owing to the toxicity and flammability of organic solvents, and the easiness of product recovery, enzymatic alcoholysis in a solvent-free system is preferable.

Immobilized *Candida Antarctica* lipase was found to be the most effective for the methanolysis of oil and fats.[4,5] However, its cost is prohibitively high for this purpose. In this paper, some efforts have been made to explore the possibility to use a relatively cheap lipase from *Thermomyces lanuginosus*, commercially called Lipozyme TL IM for biodiesel production.

Experimental

Materials. Waste oil was provided by restaurant in South China University of Technology, CRL (lipase from *Candida Rugosa*), myristic acid methyl ester, palmitic acid methyl ester, steric acid methyl ester, oleic acid methyl ester, linoleic acid methyl ester, linolenic acid methyl ester and heptadecanoic acid methyl ester were purchased from Sigma (USA), Novozym 435 (lipase from *Candida Antarctica*), Lipozyme TL IM (lipase from *Thermomyces lanuginosus*) and Lipozyme RM IM (lipase from *Rhizomucor miehei*) were kindly donated by Novo Nordisk Co. (Denmark). All other chemicals were obtained commercially and of analytical grade.

Reaction. The methanolysis of waste oil was carried out at 35℃, 130rpm using 10% immobilized lipase based on oil weight. The four-step reaction was conducted as follows. The mixture of the first-step reaction contained 10g of oil and 1:1 molar equivalent of methanol. The second-, third- and fourth-step reactions were initiated by adding 0.8:1 molar equivalent of methanol upon 90% conversion of methanol to methyl esters.

Analysis. The methyl ester (ME) content in the reaction mixture was assayed with a HP4890 gas chromatography equipped with a HP-5 capillary column (0.53mm×15m) using heptadecanoic acid methyl ester as an internal standard. The column temperature was hold at 180℃ for 1 min, raised to 186℃ at 0.8℃/min and kept for 1 min, then upgraded to 280℃ at the rate of 20℃/min.

Results and Discussion

As shown in **Table 1,** Novozym 435, Lipozyme TL IM and Lipozyme RM IM could all catalyze the reaction effectively. Lipozyme TL IM is the cheapest among them and was used for further study.

Table 1 Effect of different lipases on methanolysis of waste oil

Enzyme	ME content(%)	Methanol conversion(%)
Lipozyme TL IM	32.2	96.7
Lipozyme RM IM	32.4	97.3
Novozym 435	33.1	99.4
CRL	1.2	3.5

10g waste oil, methanol/oil molar ratio1:1, 10% lipase based on oil weight, 130rpm, 40℃, 24h.

As can be seen in **Figure 1**, the conversion dropped sharply when more than 1.5 molar equivalents of methanol were present initially in the oil mixture, suggesting the severe inactivation of the enzyme. The lipase has been proved to be irreversibly inactivated by transferring it into the fresh substrates (methanol/oil 1:1,mol/mol) and following the methanolysis course, which was in good agreement with Shimada's report.[6]

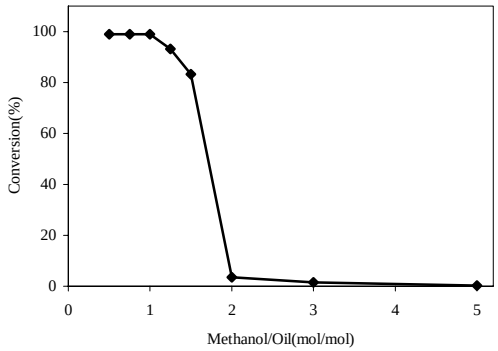

Figure 1. Effect of methanol/oil molar ratio on methanolysis of waste oil. 10g waste oil, 10% Lipozyme TL IM based on oil weight, 130rpm, 40℃, 30h. The conversion was expressed as the percentage of methanol consumed for the formation of ester (when the molar ratio of methanol /oil was less than 3), and as the ratio of methyl ester to the oil (when the molar ratio of methanol /oil was more than 3).

The influence of enzyme quantity on the methanolysis of waste oil was presented in **Figure 2**. It has been found that ME content was increased by increasing lipase quantity up to 10% based on oil weight. The conversion reached 24.7% after 12h reaction with 10% of the lipase. Interestingly, only 22.7% of waste oil was converted to its corresponding methyl esters in 12h in spite of the highest initial reaction rate with 12% enzyme. This may be explained by the observable aggregation of the immobilized enzyme at high concentration (>10% based on oil weight), which led to a lower enzymatic activity due to a higher mass transfer limitation.

Figure 3 shows temperature effect on the reaction. When the temperature was below 40℃, the reaction could be improved by raising temperature. Further increase in temperature, however, resulted in a lower ME content in the reaction mixture, indicating the inactivation of the enzyme by high temperature, which was confirmed by the low conversion of waste oil to methyl ester in the case of reusing the enzyme for the conversion of fresh substrates (methanol/oil 0.75:1, mol/mol) at 35℃.

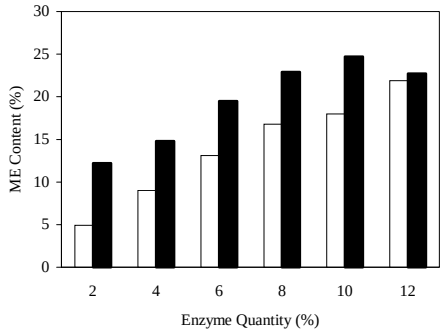

Figure 2. Effect of enzyme quantity on methanolysis of waste oil. 10g waste oil, methanol/oil molar ratio 0.75:1, 130rpm, 40℃. □, ester content upon 1h incubation; ■, ester content upon 12h incubation.

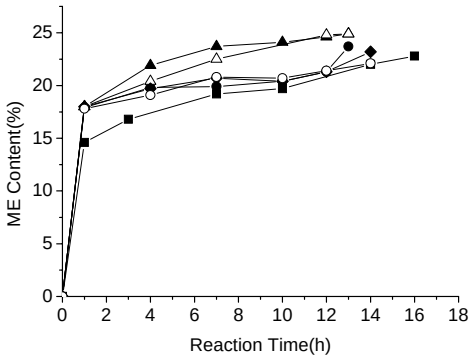

Figure 3. Effect of temperature on methanolysis of waste oil. 10g waste oil, methanol/oil molar ratio 0.75:1, 10% Lipozyme TL IM based on oil weight, 130rpm. ■,25℃;●,30℃;▲,35℃;△,40℃;◆,45℃;○,50℃.

Enzymatic methanolysis of waste oil at different shaking speed ranging from 100 to 220rpm was performed and the result showed that there was no observable difference in ME content and methanol was nearly completely converted to esters in 12h over the range from 130 to 180rpm. However, above 180rpm, little increase in reaction rate was observed with further increase in shake speed, but less ME was formed (only 22.1% in 12h at 220rpm). This was due to enzyme inactivation by higher shearing stress as indicated by the low ME content when the enzyme was reused for the methanolysis of fresh substrates (methanol/oil 0.75:1, mol/mol) at 130rpm.

At least 3 molar equivalents of methanol are required for the complete conversion of waste oil to its corresponding ME. The lipase, however, will be significantly inactivated in a mixture with more than 1 molar equivalent of methanol. Hence, a stepwise adding of methanol is necessary. The optimum substrate molar ratio (methanol/oil) was found to be 3.4:1(data not shown). The typical reaction time course was shown in **Figure 4** (with total methanol/oil molar ratio being 3.4:1). For the first-step reaction, 12h incubation (methanol/oil 1:1, mol/mol) gave a 31.9% ME content. The second step was initiated by adding methanol (with methanol/oil molar ratio being 0.8:1) into the reaction system when the incubation has lasted for 24h. The ME content reached 52.4% in 12h (total 36h). Then followed by the addition of the third batch of methanol (with methanol/oil molar ratio being 0.8:1) at the end of the second step which proceeded for 24h. The ME content reached 71.1% after incubation for another 14h (total 62h). The fourth batch of methanol (with methanol/oil molar ratio being 0.8:1) was added 10h later. The conversion of waste oil to its corresponding ME was as high as 90.2% when the incubation went on for 96h, demonstrating the effectiveness of Lipozyme TL IM, a lipase with 1,3-specificity, for biodiesel production. Acyl migration from *sn*-2 to *sn*-1 or *sn*-3 position might account for this. The similar phenomenon has also been observed by some other researchers.[7] To shorten the reaction time, the four-step procedure was modified as follows: the reaction was initiated by adding 10% immobilized enzyme (based on oil weight) into a mixture of methanol and oil (1:1, mol/mol), followed by feeding methanol three times (methanol/oil 0.8:1, mol/mol) at 12h, 24h and 38h, respectively. This four-step process converted more than 90% of the oil to its corresponding methyl esters.

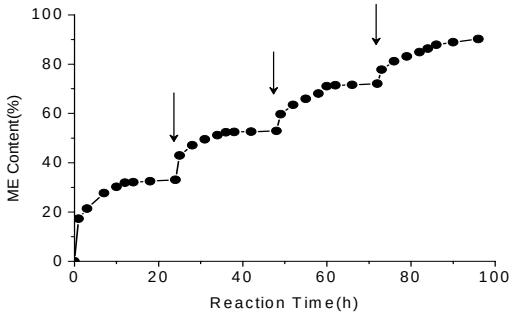

Figure 4. Reaction time course for methanolysis of waste oil. 10g waste oil, 0.38g methanol (methanol/oil molar ratio 1:1), 10% Lipozyme TL IM based on oil weight, 130rpm, 35℃. Methanol was fed three times (0.30g each) upon incubation for 24, 48 and 72h as indicated with arrows.

Conclusion

Immobilized enzyme from *Thermomyces lanuginosus*, commercially called Lipozyme TL IM showed high activity in methanolysis of waste oil. A four-step process with stepwise feeding of methanol was efficient for the conversion of waste oil to its corresponding methyl esters and so are potential for biodiesel production from waste oil.

References

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